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Host range of cotton leaf curl virus disease

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ABSTRCT : Cotton is the major commercial cash crop which greatly affects the Indian economy. Cotton leaf curl disease (CLCuD) has become a prominent and potential threat for cotton production due to its regular appearance in cotton growing belt. Availability of several alternate hosts is the main reason behind the consistent presence of its causal agent Cotton leaf curl virus (CLCuD). An experiment was performed at Cotton Section, CCS HAU, Hisar to determine the host range of CLCuD. Samples of weeds/vegetables were collected on the basis of visual CLCuD symptoms before sowing, during growing and after picking of cotton crop. Genomic DNA of the leaf samples were used for PCR amplification using a set of primers for the conserved sequence of coat protein region of several cotton leaf curl isolates. Among several samples genomic DNA of *Solenum melongina, Parthenium* spp.*, Euphorbia* spp.*, Solanum lycopercisum, Convolvulus arvensis, Aeruca sativa,Croton sprucifera* and *Lantana camara* during and before sowing of cotton*, Abutilone indicum, Achyrenthes aspera* and *Croton sprucefera* during cotton growing season and *Canabis sativa, Croton sprucifera* and *Coronopus didymus* after picking of cotton showed a positive amplified product of about 770bp. These are the host where the CLCuD present and survive before sowing of cotton, during cotton growing season and after picking of cotton crop.

Key words : Alternate hosts, coat protein, cotton leaf curl disease (CLCuD), survive

Cotton is the crop of the global importance owing to its own economic as well industrial value. China, USA, Pakistan and India are the leading cotton producing countries in the world. Several biotic stresses cause noticeable losses of cotton and among them Cotton leaf curl disease (CLCuD) is the major one.Cotton leaf curl disease substantially suppresses the cotton production in Pakistan and also the neighboring cotton-growing countries like China and India (Abbas *et al.,* 2015).

Nigeria is the place where CLCuD was first noticed on *Gossypium peruvianum* and *G. vitifolia*. The disease was first reported on *G. barbadense* in India at Indian Agricultural Research Institute (IARI), New Delhi in 1989, followed by American cotton (*G hirsutum*) in Sriganganagar district of Rajasthan in 1993. In Haryana and Punjab it was reported on *G.hirsutum* cotton in 1994, and posed a major threat to its cultivation in northern India (Yadav *et al.,* 2016). Typical symptoms of CLCuD include upward or downward leaf curling, stunted growth and 'enations' resulting in low seed cotton yield of poor quality.The disease is caused by a single stranded circular Gemini virus consisting of DNA-A and two satellites DNA that is DNA-1 and DNA beta. This virus belongs to genus *Begomovirus* and is transmitted by its exclusive vector whitefly (*Bemisia tabaci)* in circulative and persistent manner.In Pakistan and North-Western India six different types of species of

Begomoviuses were identified in cotton during the epidemic of CLCuD and many plants found to be infecting with more than one species of CLCuD (Kumar *et al.,* 2010). The CLCuD was reported as potential problem in the states of Haryana, Punjab, Rajasthan during the last one and half decade. Integrated approach by using resistant source, altered cultural practices and removal of alternate host may effective tool to proper management of CLCuD. Maharshi *et al.,* (2016) and Yadav *et al.,* (2016) screened various germplasms to find out the source of resistance against CLCuD. The CLCuV has wide host range as several alternate and collateral weeds/hosts are available alongside the crop on which the CLCuV keeps on infecting, multiplying and transfer to the cotton crop during growing season leading to the severe damage to the plant. Therefore, it is essential to find out the host range of CLCuV for proper management of its increasing inoculums year by year in North-Western India. Keeping this view experiment was conducted to determine the host range of CLCuD.

Sampling of weeds/vegetables plants : For this study, twenty six different types of weeds/vegetables samples showing CLCuD type symptoms and harboring whiteflies were collected from CCSHAU Cotton Section, Hisar and CCS HAU Cotton Research Station, Sirsa, during 2014 are listed below in Table 1.

Genomic DNA isolation : Genomic DNA of all the plant leaves was extracted by CTAB (Cetyl trimethyl ammonium bromide) method.

Qualitative and Quantitative Evaluation of DNA samples : Isolated DNA was found to have excessive poly saccharides and poly phenolics which could be used as such for molecular analysis so it was essentially processed to get poly saccharide and polyphenolics free DNA and then qualitatively and quantitatively analyzed using agarose gel electrophoresis with Lambda DNA of 150 ng/µl concentrations in 0.8 per cent agarose gel and spectrophotometry. The following Plate 1 depicts genomic DNA of some of samples selected for molecular analysis. The quality was found to be fairly good for further processing. Samples having DNA concentration >150 ng were used further.

Amplification of cotton leaf curl virus coat protein specific gene sequences : PCR technique was used to amplify CLCuV coat protein gene from genomic DNA of different weeds/vegetables plants. For this purposeone pair of coat protein gene specific primer G1/C3 (5'TAATATCAATTCGTTACAGAG3'/ 5'AATTATGTCGAAGCGAGCTG3') (Kumar *et al.,* 2010) of cotton leaf curl virus (CLCuV) (published in literature) were got synthesized from Bioserve Pvt. Ltd. Hyderabad and used in the present study.A final volume of 15ul PCR mix containing 1µl (100ng) DNA template (isolated from the infected leaf tissues), 1.15 µl (1.15U) Taq DNA polymerase, 1.5µl (1mM) dNTPs, 0.75 µl (5pM) of each primer and remaining sterilize nuclease free water was taken in a PCR tube. (PCR condition used was 28 cycles with 92°C for 3 min (Initial denaturation), 91°C for 45 seconds (Denaturation), 43.5°C for 45 seconds (annealing), and extension for 2 min at 72°C and final extension for 5 min at 72°C. Amplified products were resolved by submerged horizontal electrophoresis in 2.0 per cent (w/v) agarose gel

and visualized by staining with ethidium bromide. PCR amplification products were viewed under UV light and photographed using Biorad Gel Documentation System.

Viral disease makes up 47 per cent of the new emerging diseases to the plants so, through this study we can say the plant infection capability of viruses is very successful. Cotton leaf curl viral disease is also very potential emerging threat to cotton cultivation in north western India. Availability of alternate host upto a great extent throughout the year is the major responsible factor for efficient viral survival in this region. In case of WTGs (Whitefly transmitted Geminiviruses), the gene encoding the coat protein is generally homologous. Therefore, coat protein gene has traditionally proven useful for plant virus identification and classification. The utility of coat protein to identify field isolates, weed species are reported by several scientists. Our result comprises the hosts on which CLCuV is able to infect and multiply before cotton growing period, during growing season and after picking of cotton crop using primer specific to coat protein gene of CLCuV.

Twenty four DNA samples of weeds/ vegetables collected before sowing of cotton were amplified using CtLCV#33 primer but only eight samples of different hosts *viz.Solenum melongina, Parthenium* spp., *Euphorbia* spp., *Solanum lycopercisum, Convolvulus arvensis, Aeruca sativa, Croton sprucifera* and *Lantana camara* were found positive amplification. Plate 2, 3 shows documentation of PCR amplification pattern obtained by electrophoretically resolved polymerisation. The amplified PCR product of about 770bpdetermines the presence of CLCuV.

Among eighteen different weeds/ vegetables collected during cotton growing season only three samples of different hosts *viz. Abutilone indicum, Achyrenthes aspera,* and *Croton sprucifera* were found the amplification of about 770bp (Plate 4). These are the host having CLCuVgenomic DNA along with own genomic DNA.

After picking of cotton fourteen various weeds/vegetables host were collected andDNA was subjected to PCR amplification. Among all samples three different hosts *viz.Canabis sativa,*

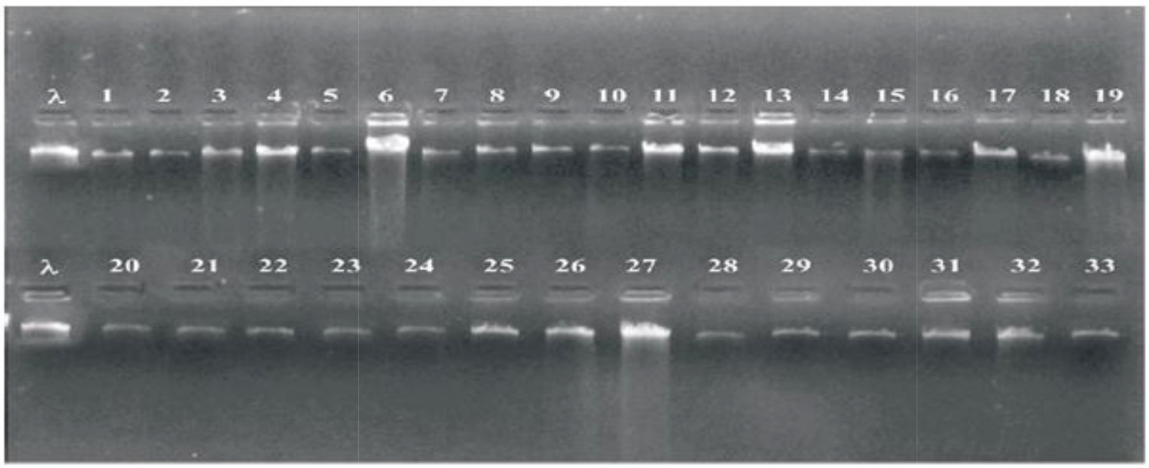

Plate 1: Genomic DNA of weeds/vegetables collected for determining presence of CLCuV

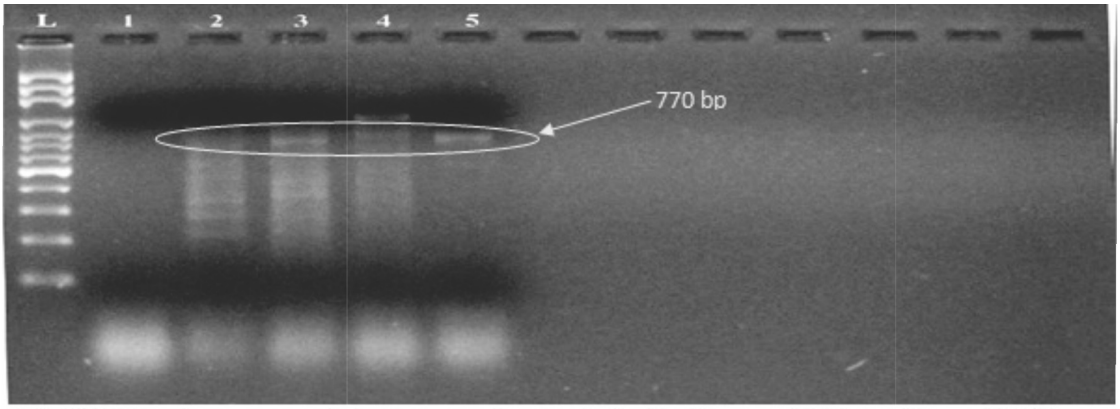

Plate 2 1. *Capsicum annuum* 2. ***Solenum melongina*** 3. ***Parthenium spp.*** 4. ***Euphorbia spp.*** 5. ***Solenum lcopersicum***

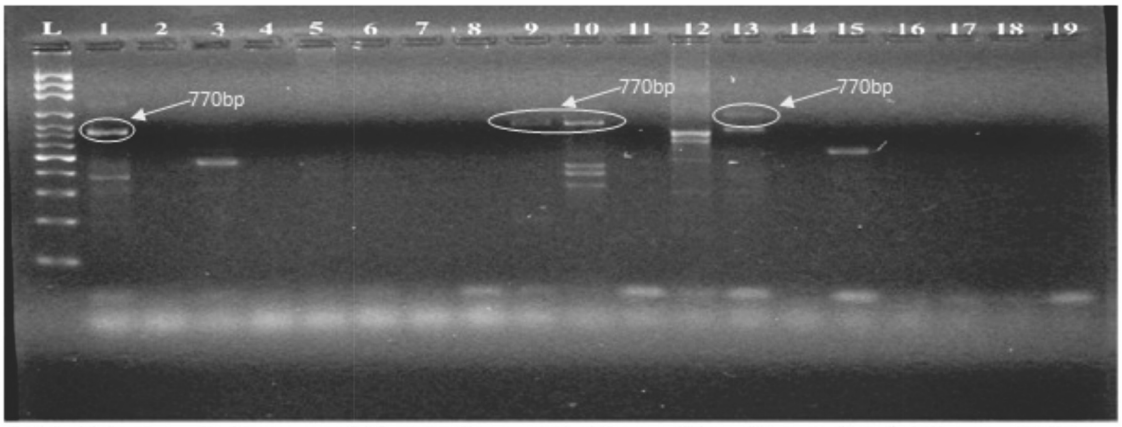

Plate 3 1. ***Convolvulus arvensis*** 2. *Abutilon spp.* 3. *Chenopodium album* 4. *Parthenium spp.* 5. *Aeruca persica* 6. *Convolvulus arvensis* 7. *Parthenium spp.* 8. *Ameranthus spp.* 9. ***Aeruca sativa*** 10. ***Croton sprucifera*** 11. *Morus alba* 12. *Tribulus terrestris* 13. ***Lantana camara*** 14. *Abutilon spp.* 15. *Solanum melongina* 16. *Parthenium spp.* 17. *Solenum melongina* 18. *Euphorbia spp.* 19. *Clerodendron enarmi*

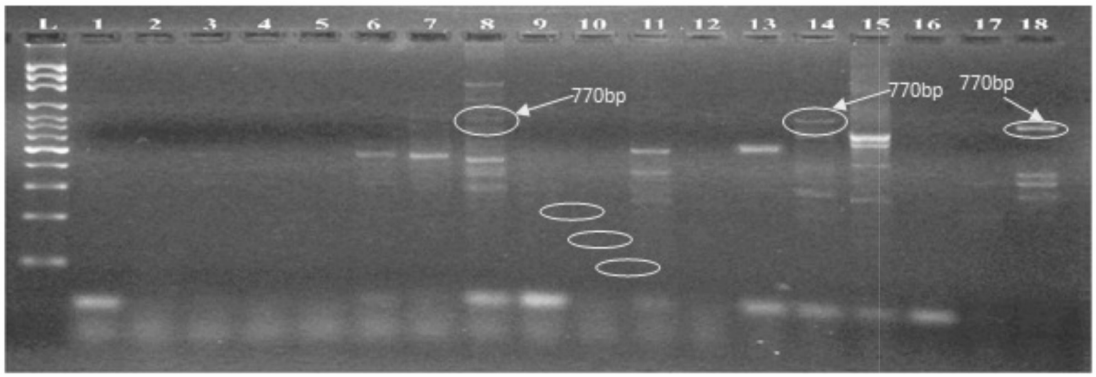

Plate 4 1. *Abutilon indicum* 2. *Abutilon spp.* 3. *Chinopodium album* 4. *Canabis sativa* 5. *Convolvulus arvensis* 6. *Chinopodium album* 7. *Aeruca persica* 8. ***Abutilon indicum*** 9. *Lanatana camara* 10. *Parthenium spp.* 11. *Withania somanifera* 12. *Aeruca persica* 13. *Solanum lycopersicum* 14. ***Achyrenthus aspera*** 15. *Tribulus terresteris* 16. *Clerodendron enarmi* 17. *Chenopodium murale* 18. ***Croton sprcifera***

Table 1. Samples of weeds/ vegetables collected for determining presence of CLCuV

S.No.	Before sowing	During crop season	After harvesting
1.	Amranthus*(Amerenthus spp.)*	-	-
2.	-	-	Ageratum (*Ageratum conyzoides)*
3.	-	Ashwagandha (*Withoniasomanifera*)	-
4.	-	-	Badi dudhi (*Euphorbia spp.)*
5.	Bathua*(Chenopodium album)*	Bathua*(Chenopodium album)*	Bathua*(Chinopodium album)*
6.	Bhakri*(Tribulus terrestris)*	Bhakri*(Tribulus terrestris)*	-
7.	-	Bhang (*Cannabis sativa*)	Bhang (*Cannabis sativa*)
8.	Brinjal *(Solenummelongina)*	-	-
9.	Bui (*Aeruca sativa*)	-	-
10.	Chilli *(Capsicum annuum)*	-	Chilli (C*apsicum annuum*)
11.	Choti dudhi *(Euphorbia spp)*	-	Choti dudhi *(Euphorbia spp)*
12.	Clerodendron*(Clerodendronenarmi)*	Clerodendron*(Clerodendronenarmi)*	-
13.	Congress grass *(Parthenium spp)*	Congress grass (*Parthenium spp*)	Congress grass (*Parthenium spp*)
14.	Hirankhuri*(Convolvulus arvensis)*	Hirankhuri*(Convolvulus arvensis)*	-
15.	-	-	Ghobisarson (*Brasiccanapus sub sp. Olerifera var. Napus*)
16.	Janglimirch *(Croton sprucifera)*	Janglimirch *(Croton sprucifera)*	Janglimirch *(Croton sprucifera)*
17.	Kali bue *(Aerucapersica)*	Kali bue *(Aerucapersica)*	Kali bue *(Aerucapersica)*
18.	Kangibunti*(Abutiloneindicum)*	Kangibunti*(Abutiloneindicum)*	-
19.	-	Kharbathu (*Chenopodium mural)*	-
20.	Lantana camara *(Lantana camara)*	Lantana camara *(Lantana camara)*	-
21.	Pili bunti *(Abutilone spp.)*	Pili bunti *(Abutilone spp.)*	Pili bunti *(Abutilone spp.)*
22.	-	-	Peet papra (*Cronopusdidymus)*
23.	-	Puthkanda(*Achyrenthes aspera*)	-
24.	Shatoot *(Morus alba)*	-	-
25.	Tomato *(Solanumlycopersicum)*	Tomato *(Solanum lycopersicum)*	Tomato *(Solanum lycopersicum)*
26.	-	-	Verneasineria (*Verneasineria*)

Croton sprucifera and*Coronopus didymus* were found the positive amplification of about 770bp coat protein gene (Plate 5).It is concluded that *Solenum melongina, Parthenium* spp., *Euphorbia spp., Solanum lycopercisum, Convolvulus arvensis,Aeruca sativa,Croton sprucifera, Lantana camara, Abutilone indicum, Achyrenthes aspera,Canabis sativa* and*Coronopus didymus* is the host range of CLCuD plays an important role in causing this virus to spread over cotton crop. They provide inoculum to the vector for transmitting the same in cotton. There are many ornamental and common weeds plants which have been found infected with whitefly-transmitted Geminiviruses. Reports appearance of the CLCuD on the *Sidasps, Abutilon indicum, Hibiscus rosa-sinensis, and Althea roses* on the basis of visual symptoms. In *Phaseolus vulgaris*, pepper, tomato and tobacco, transmission studies and ELISA showed the presence of CLCuD.

Primary inoculums present during offseason in the form of weeds and other host has potential role in spread of cotton leaf curl disease. conducted transmission studies on Okra, Hollyhock, Tomato, *Physalis floridana*,

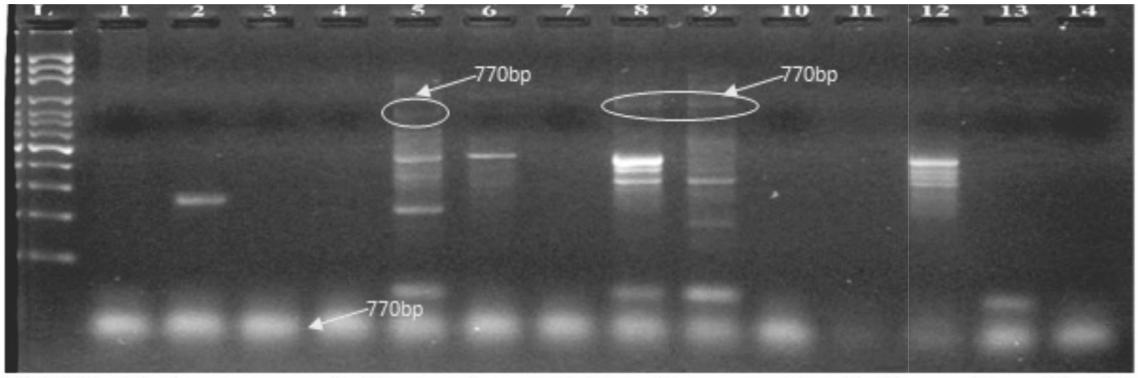

Plate 5 1. *Capsicum annuum* 2. *Vernea cineraria* 3. *Ageratum conyzoides* 4. *Chenopodium album* 5. ***Canabis sativa*** 6. *Euphorbia spp.* 7. *Parthenium spp.* 8. ***Croton sprucifera*** 9. ***Cornopus didymus*** 10. *Brassica napus* sub sp. *Oleifera* var. *napus* 11. *Aeruca persica* 12. *Abutilon spp.* 13. *Solanum lycopersicum* 14. *Euphorbia spp.*

Nicotiana benthamiana and French bean to detect the presence of cotton leaf curl virus. DNA probe hybridization has been conducted on Hollyhock, *Sida ageratum*, China rose to detect the presence of CLCuD.*Tribulus terrestris* and *Cucumus sp.* have shown the presence of DNA-A and Beta DNA of CLCuD in their genome. *Chorchorusacutangularis*, *Melilotusindica*, *Ageratum conyzoides* were detected CLCuD positive on the basis of DNA-A and DNA beta probe hybridisation. In National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad, the following plants have been confirmed to be the host of CLCuV through PCR detection test these are*Abelmoscusesculentus* (Okra), *Hibiscus cannabinus* (Sunkukra), *Hibiscus rosa- sinensis* (China rose), *Hibiscus tiliaceus (Saklai)*, *Ageratum sp.* and *Sidaalba. Sida spp., Achyranthus spp., Clearodeadron spp.* have been confirmed to be host of CLCuD using coat protein gene amplification. Similarly, Monga *et al.*, (2011) reported the *Convolvulus arvensis, Capsicum* spp., *Pathenium* spp., *Solanum nigrum, Digeria arvensis, Lantana camara, Achyranthus aspera, Chenopodium album, Spinaceasps., Xanthium strumarium* as the host of CLCuV using coat protein gene amplification. Conclusivelyalternate hosts are most determinative role in spread and availability of cotton leaf curl virus during off season. It is essential to determine its host range to regulate the increasing inoculum as well as proper management of cotton leaf curl disease.

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